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Secrets of the Cutaneous Basement Membrane

Sarolta Karpati[1]

The paper in this issue by Has and co-workers reports 15 non-Herlitz epidermolysis bullosa patients with the same single amino-acid substitution in collagen XVII, all of whom presented with clinical and pathological features resembling Kindler syndrome. Here we consider why and how a hemidesmosomal pathology can mimic a focal adhesion bond disease, both clinically and ultrastructurally.

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In this issue of *JID,* Cristina Has and an international group of specialists on epidermolysis bullosa (EB) report on a substitution mutation of collagen XVII that is associated with several unexpected skin and mucosal features that are ordinarily not observed in patients with junctional EB other (non-Herlitz-type junctional EB) disease. The authors also find a clinical overlap with another inherited EB, the Kindler syndrome. Furthermore, the clinical features developed rather late, years and even decades after birth in most of the patients, indicating a long-lasting mechanism of functional compensation and slow progression of the inherited basement membrane (BM) damage.

Structural stability and dynamics of collagen XVII within the hemidesmosomal anchoring complex

The collagen XVII (180 kDa) was identified within the cutaneous basement membrane zone (BMZ) chronologically as the second, and functionally as the major, autoantigen of bullous pemphigoid (BP), and therefore it has also been called BPAG2 (BP antigen 2) or BP180. The final product is a trimer of three 180 kDa α1 XVII chains [α1(XVII)3], and it anchors basal keratinocytes at the hemidesmosomal plaque through the lamina lucida to the lamina densa of the BM. Its intracellular N-terminal domain contributes to hemidesmosomal plaque stability, and it interacts with the plaque proteins plectin and BPAG1 (BP230) and also with the intracellular tail of β4 integrin. The 120 kDa extracellular, flexible, collagenous ectodomain of the molecule is part of the anchoring filament and, while binding to the α6 integrin and laminin 332, crosses the lamina lucida of the BM where it anchors the lamina densa. This ectodomain contains 15 collagenous subdomains separated by 16 non-collagenous (NC) interruptions (Birk and Bruckner, 2011).

The 120 kDa ectodomain is shed from the 180 kDa transmembrane molecule at the external surface of basal keratinocytes within the NC16 domain by metalloproteinases of the ADAM (A Disintegrin And Metalloproteinase) family, predominantly by the tumor necrosis factor-converting enzyme (TACE alias ADAM 17) or by ADAM 9 or ADAM 10. The shed ecto-collagen XVII functions as a ''mobile'' component of the extracellular matrix (ECM); it is present not only in the lamina lucida but also in the uppermost dermal connective tissue. The ectodomain acquires new epitopes through proteolytic cleavage, which identify and distinguish the cleaved molecules from the uncleaved 180 kDa protein. There are also smaller shed ectodomains (e.g., 95 kDa) with distinct cleavage sites and neoepitopes. Neoepitopes within the NC16A domain are recognized preferentially in autoimmune blistering diseases such as BP, pemphigoid gestationis, and the linear IgA dermatosis (Nishie *et al.,* 2010).

Ectodomain shedding weakens basal keratinocyte binding to the underlying BM when the architectural stability of the molecule is disrupted. Shedding may be of physiological importance during the differentiation of the basal keratinocytes, as well as in their movement during wound healing and tissue regeneration (Hashmi and Marinkovich, 2011).

Cross-talk along the BMZ: the key role of β-integrins in adhesome interactions

The bidirectional interaction between cells and their environment is mediated predominantly via integrins that accumulate in adhesomes. There are collagen-, laminin-, and leukocyte-binding integrins. The ability of integrins to be activated and to initiate signaling pathways depends upon the type of adhesion complexes that are formed between cells and the ECM. Activated integrins become clustered and form dynamic integrin adhesomes, which are ''signaling points'' (Schiller and Fässler, 2013). Proteins that bind the intracellular domain of β-integrins are able to activate these β-integrins and to increase their adhesion to the ECM. β1 integrins, the largest integrin subfamily, are usually involved in cell migration, proliferation, and differentiation, and they participate in development, tissue homeostasis, and tumor progression. Integrin-targeted biological therapies can be very effective tools for tumor suppression (Marelli *et al.,* 2013).

Recent studies have demonstrated how in genetically engineered cells diff-

[1]*Department of Dermatology, Venereology and Dermato-oncology, Semmelweis University, Budapest, Hungary*

Correspondence: Sarolta Karpati, Department of Dermatology, Venereology and Dermato-oncology, Semmelweis University, Maria Utca 41, Budapest 1085, Hungary. E-mail: skarpati@t-online.hu

Clinical Implications

- A rare form of junctional epidermolysis bullosa presented with clinical symptoms resembling Kindler syndrome occurs in patients carrying the p.R1303Q mutation of collagen XVII.
- This arginine–glutamine substitution seems to induce a functional damage in the basement membrane and in the underlying extracellular matrix, causing slowly progressive structural changes manifested as atrophy, scarring or acral sclerodermiform changes, loss of dermatoglyphs, nail dystrophy, and mild skin fragility with variable mucosal involvement.
- An international group of specialists collected clinical, ultrastructural, and antigen mapping data on the skin from 15 patients carrying this mutation.
- The pathological significance of the new glutamine in the non-collagenous extracellular domain of collagen XVII remains unclear, however.

erent integrins can cooperate to modulate the signaling pathways that are required for adhesion morphology, cytoskeleton distribution, cell contractility, and rigidity sensing of the ECM. Integrin classes have been shown to be capable of forming unique signaling centers consisting of adaptor and signaling molecules. These are able to initiate distinct signaling pathways, and thus cells can ultimately translate even mechanical signals into biochemical changes (Schiller *et al.,* 2013).

α3β1 Integrins and kindlins in focal adhesions

Focal adhesions of the basal keratinocytes are formed primarily along a core of transmembrane α3β1 integrins that connect the actin cytoskeleton to the BMZ and serve as adhesion receptors in bidirectional ECM–cell interactions. These adhesomes may incorporate more than 100 different proteins around the dynamically formed group of α3β1 heterodimers. These integrins, as transmembrane bidirectional signaling molecules, transduce extracellular signals into cells upon extracellular ligand binding, and, by recruiting signaling and adaptor proteins to their intracellular tails, they initiate actin reorganization and intracellular signaling pathways. Kindlins are a family of cytoplasmic adaptor proteins localized adjacent to focal adhesions (Meves *et al.,* 2009). All kindlins contain a central FERM domain (band 4.1, ezrin, radixin, moesin), and they are able to bind and activate β-integrins and participate in cell–matrix cross-talk via in–out and out–in signaling. In the skin, Kindlin-1 is present along the focal adhesions of the dermo–epidermal junction where they bind to the cytoplasmic tails of β1- and β3-integrins. Mutations in the Kindlin-1 (FERMT1) gene have been found to cause the Kindler syndrome, a type of EB disease (Bruckner-Tuderman and Has, 2013).

α6β4 Integrins in hemidesmosomes

Collagen XVII binds the cytoplasmic tail of β4 integrin and, indirectly through the hemidesmosomal plaque proteins (plectin and BP230) to the keratin filament network. Collagen XVII and α6 integrins interact with each other and with laminin 332 within the BM.

Mutations in collagen XVII, laminin 332, and integrin α6β4 induce a heterogenous group of inherited skin fragility diseases called junctional EB other (Kiritsi *et al.,* 2013).

Disturbed signaling and cell adhesion in collagen XVII pathologies

- *In vitro* studies using collagen XVII-deficient and -sufficient epidermal keratinocyte cultures suggest that this transmembrane collagen may have a central role in the initiation of epithelial inflammatory responses (Van den Bergh *et al.,* 2012).
- The role of the ADAM family in collagen XVII shedding also suggests direct and/or indirect contributions of BPAG2 to cell–matrix signaling. These proteinases generally initiate cell migration and ligand–receptor interactions: ADAMs also mediate ectodomain shedding initiated by cytokines and their receptors, and they regulate signaling during inflammatory responses via tumor necrosis factor, IL-6, and EGF receptors and Notch (Khokha *et al.,* 2013).
- Extracellular phosphorylation represents a novel way of regulating cell adhesion, cell proliferation, and immune responses. Extracellular phosphorylation of collagen XVII by ecto-casein-kinase 2 inhibits the ectodomain shedding by TACE and, through that, it modifies adhesion and motility (Zimina *et al.,* 2007).
- Recently, transmembrane collagens were identified in the brain as active molecules with dynamic functions during neuronal and synaptic differentiation, axonal outgrowth, and brain development. The presence of and autoantibody binding to brain collagen XVII was correlated with enhanced CNS pathology in patients with BP (Seppänen, 2013).

The story of the arginine–glutamine substitution *COL17A1* p.R1303Q and the pathological reorganization of the cutaneous BMZ

The cutaneous BMZ is a highly complex suprastructure functioning between the epidermis and the dermis as a static bridge that provides resistance against mechanical forces and as a dynamic connection that provides tools for bilateral cross-talk, signaling, and cellular trafficking between the ECM and basal keratinocytes (Hashmi and Marinkovich, 2011; Tsuruta *et al.,* 2011). Laminin 332 is thought to be a supramolecular bridge communicating with the integrins α6β4 at hemidesmosomes and also with α3β1 integrins within the focal adhesions (Kiritsi *et al.,* 2013). The base of the BMZ suprastructure is built primarily from two networks superimposed and welded together by perlecan: the laminin 332 network in the lamina lucida and the collagen IV network within the lamina densa (Behrens *et al.,* 2012).

The *COL17A1*p.R1303Q hemidesmosome-anchoring filament complex-bound mutation is interesting as it seems to disturb adhesive BMZ activities only modestly (for years as compensated

mild skin fragility), but it appears to extend the effects to the functions of focal adhesions (to begin resembling Kindler's disease). Furthermore, this mutation progressively reorganizes the ultrastructure of the BMZ, as indicated by the loss of hemidesmosomes and the loss of lamina lucida, while the anchoring fibrils become covered with an amorphous material. Laminin 2, collagen IV, and collagen VII staining showed a broad and irregular distribution along the BMZ and within the underlying papillary dermis.

The authors speculate that the R1303Q mutation, with a new glutamine residue at the extracellular NC domain 4 of collagen XVII, might be a new binding site for tissue transglutaminase (TG2), an enzyme involved in connective tissue cross-linking (Lai and Greenberg, 2013). The observation that in patients with the R1303Q mutation both TG2 and tenascin C showed a pathological distribution along and under the BMZ supports this hypothesis.

Conclusion

The cause of R1303Q-induced pathology remains uncertain, but it is definitely of considerable interest. The very complex BMZ unit tends to keep its secrets, but the paper by Has *et al.* (2014) now shows us how important new information can be gained about this supramolecular system by careful phenotype analysis of related diseases. On the other hand, there is still much to be done.

> Has *et al.* now show how new knowledge on the BMZ arose from careful analyses of disease phenotype.

Several of the questions summarized by Fässler and his group recently as ''tasks'' for ECM researchers include the following (Schiller and Fässler, 2013):

Adhesome interactions—how is the formation of a cell–matrix adhesion initiated?

What are the dynamics of protein–protein interactions at cell–matrix adhesion sites, and what is the structure of these supramolecular assemblies?

Mechanotransduction—how is mechanical force translated into biochemical signals at cell–matrix adhesions? How are signals from cell–matrix adhesions spatially propagated to the cytoskeleton? How does mechanical tension trigger the recruitment of proteins to cell–matrix adhesion sites?

Signaling, specificity, and diversity—how diverse is cell–matrix adhesion signaling in different cell types and tissues? Which proteins of the adhesome mediate integrin-subtype-specific functions? What are the molecular mechanisms of integrin cross talk? Where and how are integrin and growth factor receptor signals combined?

CONFLICT OF INTEREST

The author states no conflict of interest.

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